What is claimed is:

1. A purified polynucleotide comprising a nucleotide sequence encoding a polypeptide that comprises a fragment of a human Asp1 protein (hu-Asp1),

wherein said polynucleotide lacks nucleotide sequence encoding at least one portion of the hu-Asp1 protein, selected from the group consisting of (a) the transmembrane domain of said hu-Asp1 protein; and (b) the amino-terminal propeptide of said hu-Asp1 protein; and

wherein the polypeptide encoded by said polynucleotide retains amyloid precursor protein (APP) proteolytic activity characteristic of said human Asp1 protein.

2. A polynucleotide according to claim 1, wherein the polypeptide has hu-Asp1 α-secretase activity.

3. A polynucleotide according to claim 1, wherein the polypeptide has hu-Asp1 β-secretase activity.

4. A polynucleotide according to claim 1, wherein said polynucleotide lacks nucleotide sequence encoding the transmembrane domain of said hu-Asp1 protein.

5. A polynucleotide according to claim 4, wherein the polynucleotide comprises a nucleotide sequence that encodes a fragment of hu-Asp1 having the amino acid sequence set forth as SEQ ID NO: 2, and wherein the polynucleotide lacks sequence encoding the transmembrane domain amino acids 469-492 of SEQ ID NO: 2.

6. A polynucleotide according to claim 5 wherein the polynucleotide further lacks nucleotide sequence encoding the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

7. A polynucleotide according to claim 6, wherein said polynucleotide further lacks nucleotide sequence encoding amino acids 1-62 of SEQ ID NO: 2.

8. A polynucleotide according to claim 1, wherein said polynucleotide lacks nucleotide sequence encoding the amino-terminal propeptide of said hu-Asp1 protein.

9. A polynucleotide according to claim 8, wherein the polynucleotide comprises a nucleotide sequence that encodes a fragment of hu-Asp1 having the amino acid sequence set forth as SEQ ID NO: 2, and wherein the polynucleotide lacks sequence encoding the signal peptide and amino terminal propeptide amino acids 1-62 of SEQ ID NO: 2.

10. A vector comprising the polynucleotide of claim 1.

11. A host cell transformed or transfected with a vector of claim 10.

12. A host cell transformed or transfected with a polynucleotide of claim 1.

13. A polynucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to a nucleic acid comprising the complement of the nucleotide sequence set forth in SEQ ID NO: 1, wherein the polynucleotide encodes a polypeptide having amyloid precursor protein (APP) processing activity, and

wherein said polynucleotide lacks nucleotide sequence encoding a transmembrane domain and said encoded polypeptide lacks a transmembrane domain.

14. A polynucleotide comprising a nucleotide sequence that hybridizes under stringent conditions to a nucleic acid comprising the complement of the nucleotide sequence set forth in SEQ ID NO: 1, wherein the polynucleotide encodes a polypeptide having amyloid precursor protein (APP) processing activity, and

wherein said polynucleotide lacks nucleotide sequence encoding a propeptide and said encoded polypeptide lacks a propeptide.

15. A purified polypeptide that comprises a fragment of a human Asp1 protein (hu-Asp1),

wherein said polypeptide lacks at least one portion of the hu-Asp1 protein selected from the group consisting of (a) the transmembrane domain of said hu-Asp1 protein; and (b) the amino-terminal propeptide of said hu-Asp1 protein; and

wherein the polypeptide retains amyloid precursor protein (APP) proteolytic activity characteristic of said human Asp1 protein.

16. A polypeptide according to claim 15, wherein the polypeptide has hu-Asp1 α-secretase activity.

17. A polypeptide according to claim 15, wherein the polypeptide has hu-Asp1 β-secretase activity.

18. A polypeptide according to claim 15, wherein said polypeptide lacks the transmembrane domain of said hu-Asp1 protein.

19. A polypeptide according to claim 18, wherein the polypeptide comprises a fragment of hu-Asp1 having the amino acid sequence set forth as SEQ ID NO: 2, and wherein the polypeptide lacks transmembrane domain amino acids 469-492 of SEQ ID NO: 2.

20. A polypeptide according to claim 19 which further lacks cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

21. A polypeptide according to claim 20, wherein said polypeptide further lacks amino acids 1-62 of SEQ ID NO: 2.

22. A polypeptide according to claim 15 that lacks the amino-terminal propeptide of said hu-Asp1 protein.

23. A polypeptide according to claim 22 that further lacks the signal peptide of the hu-Asp1 protein.

24. A polypeptide according to claim 23 that comprises a fragment of hu-Asp1 having the amino acid sequence set forth as SEQ ID NO: 2, wherein the polypeptide lacks signal peptide and amino terminal propeptide amino acids 1-62 of SEQ ID NO: 2.

25. A polypeptide comprising an amino acid sequence at least 95% identical to a fragment of the hu-Asp1 protein having the amino acid sequence of SEQ ID NO: 2,

wherein said polypeptide lacks at least a transmembrane domain or an amino-terminal propeptide characteristic of a hu-Asp1 protein; and

wherein the polypeptide has amyloid precursor protein (APP) proteolytic activity characteristic of said human Asp1 protein.

26. A method of identifying agents that modulate amyloid precursor protein (APP) processing activity of human hu-Asp1 aspartyl protease (hu-Asp1), comprising steps of:

(a) contacting amyloid precursor protein (APP) and purified and isolated hu-Asp1 in the presence and absence of a test agent;

(b) determining APP processing activity of the hu-Asp1 in the presence and absence of the test agent; and

(c) identifying agents that modulate APP processing activity of hu-Asp1 by comparing the APP processing activity of the hu-Asp1 in the presence and absence of the test agent, wherein reduced activity in the presence of the test agent identifies an agent that inhibits hu-Asp1 activity and increased activity in the presence of the test agent identifies an agent that enhances hu-Asp1 activity.

27. A method according to claim 26 , wherein the hu-Asp1 comprises a polypeptide purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes hu-Asp1.

28. A method according to claim 27 wherein the nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2;

(b) a nucleotide sequence encoding a fragment of hu-Asp1 (SEQ ID NO: 2), wherein said fragment exhibits aspartyl protease activity characteristic of hu-Asp1;

(c) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a hu-Asp1-encoding polynucleotide (SEQ ID NO: 1).

29. A method according to claim 27, wherein the nucleotide sequence encodes a hu-Asp1 amino acid sequence lacking transmembrane amino acids 469-492 of SEQ ID NO: 2.

30. A method according to claim 29, wherein the nucleotide sequence encodes a hu-Asp1 amino acid sequence further lacking the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

31. A method according to claim 30, wherein the nucleotide sequence encodes a hu-Asp1 amino acid sequence further lacking amino terminal amino acids 1-62 of SEQ ID NO: 2.

32. A method according to claim 26 wherein the step (b) comprises determining an α-secretase APP processing activity of the hu-Asp1 protein.

33. A method according to claim 26 wherein the determining step comprises measuring the production of amyloid alpha peptide by the cell in the presence and absence of the test agent.

34. A method according to claim 26 wherein step (b) comprises determining a β-secretase APP processing activity of the hu-Asp1 protein.

35. A method according to claim 26 wherein the determining step comprises measuring the production of amyloid beta peptide by the cell in the presence and absence of the test agent.

36. A method according to claim 26, further comprising a step of treating Alzheimer's Disease with an agent identified as a modulator of APP processing activity of hu-Asp1 according to steps (a)-(c).

37. A method for identifying agents that modulate the amyloid precursor protein (APP) processing activity of human Asp1 aspartyl protease (hu-Asp1), comprising the steps of:

(a) contacting hu-Asp1 and amyloid precursor protein (APP) in the presence and absence of a test agent, wherein the contacting comprises growing a host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence encoding the hu-Asp1 in the presence and absence of the test agent;

(b) determining the APP processing activity of the hu-Asp1 in the presence and absence of the test agent; and

(c) identifying an agent that modulates APP processing activity by comparing the APP processing activity of the hu-Asp1 polypeptide in the presence of the test agent to the activity in the absence of the test agent, wherein reduced activity in the presence of the test agent identifies an agent that inhibits hu-Asp1 APP processing activity and increased activity in the presence of the test agent identifies an agent that enhances hu-Asp1 APP processing activity.

38. A method according to claim 37, wherein the determining step comprises assaying for cleavage of APP at the α-secretase processing site.

39. A method according to claim 38 wherein the determining step comprises measuring the production of amyloid alpha peptide by the cell in the presence and absence of the test agent.

40. A method according to claim 37, wherein the determining step comprises assaying for cleavage of APP at the β-secretase processing site.

41. A method according to claim 37, wherein the host cell expresses APP.

42. A method according to claim 37, wherein the host cell expresses an APP having an amino acid sequence that includes a carboxy-terminal di-lysine.

43. A method according to claim 37, wherein the host cell expresses as APP comprising the Swedish mutation (K→ N, M→L) adjacent to the β-secretase processing site.

44. A method according to claim 37 wherein the determining step comprises measuring the production of amyloid beta peptide by the cell in the presence and absence of the test agent.

45. A method according to claim 37 wherein the nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2;

(b) nucleotide sequence encoding a fragment of hu-Asp1 (SEQ ID NO: 2), wherein said fragment exhibits aspartyl protease activity characteristic of hu-Asp1; and

(c) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a hu-Asp1-encoding polynucleotide (SEQ ID NO: 1).

46. A method according to claim 37 wherein the host cell comprises a vector that comprises the polynucleotide.

47. A method according to claim 37, further comprising a step for treating Alzheimer's Disease with an agent identified as a modulator of hu-Asp1 according to steps (a)-(c).

48. A method for identifying agents that modulate the APP processing activity of hu-Asp1 asparty protease, comprising the steps of:

(a) contacting an hu-Asp1 aspartyl protease and amyloid precursor protein (APP) in the presence and absence of a test agent, wherein the hu-Asp1 aspartyl protease is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a hu-Asp1-encoding polynucleotide set out as SEQ ID NO: 1;

(b) determining the APP processing activity of the hu-Asp1 aspartyl protease in the presence and absence of the test agent; and

(c) comparing the APP processing activity of the hu-Asp1 aspartyl protease in the presence of the test agent to the activity in the absence of the agent to identify agents that modulate the activity of the hu-Asp1 aspartyl protease, wherein a modulator that is an hu-Asp1 inhibitor reduces APP processing and a modulator that is an hu-Asp1 agonist increases such processing.

49. A method of claim 48, wherein the hu-Asp1 aspartyl protease is purified and isolated.

50. A method of claim 48, further comprising a step of treating Alzheimer's Disease with an agent identified as an inhibitor of hu-Asp1 according to steps (a)-(c).

51. A method of claim 48, wherein the APP processing activity of hu-Asp1 is cleavage of APP peptide within the α-secretase processing site.

52. A method of claim 48, wherein the APP processing activity of hu-Asp1 is cleavage of APP peptide at the β-secretase processing site.

53. A method for assaying hu-Asp1 α-secretase activity comprising the steps of:

(a) contacting hu-Asp1 enzyme with an amyloid precursor protein (APP) substrate, wherein said substrate contains an α-secretase cleavage site; and

(b) measuring cleavage of the APP substrate at the α-cleavage site, thereby assaying hu-Asp1 α-secretase activity.

54. A method according to claim 53, wherein the hu-Asp1 enzyme comprises a polypeptide produced in a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes hu-Asp1 or a fragment thereof that retains Asp1 α-secretase activity.

55. A method of claim 54, wherein the hu-Asp1 enzyme is purified and isolated from said cell.

56. A method according to claim 54, wherein the nucleotide sequence encodes a polypeptide that comprises the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, wherein said fragment retains α-secretase activity.

57. A method according to claim 54, wherein the polynucleotide sequence encodes a hu-Asp1 amino acid sequence lacking the transmembrane amino acids 469-492 of SEQ ID NO: 2.

58. A method according to claim 57, wherein the polynucleotide sequence encodes a hu-Asp1 amino acid sequence further lacking the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

59. A method according to any one of claims 55, wherein the hu-Asp1 lacks amino terminal amino acids 1-62 of SEQ ID NO: 2.

60. A method according to claim 53 wherein the contacting step comprises growing a cell transfected or transformed with a polynucleotide encoding hu-Asp1 enzyme or a fragment thereof that retains hu-Asp1 α-secretase activity, wherein the cell is grown under conditions in which the cell expresses the hu-Asp1 enzyme in the presence of the APP substrate.

61. A method of claim 60, wherein said cell expresses a polynucleotide encoding an APP substrate containing an α-secretase cleavage site, and wherein the contacting step comprises growing the cell under conditions in which the cell expresses the hu-Asp1 enzyme and the APP substrate.

62. A method of claim 53, wherein the APP substrate α-secretase cleavage site comprises the amino acid sequence LVFFAEDF or KLVFFAED.

63. A method of claims 62, wherein the APP substrate comprises a detectable label.

64. A method of claim 53, wherein the detectable label is selected from the group consisting of radioactive labels, enzymatic labels and flourescent labels.

65. A method of claim 53, wherein the APP substrate comprises a human APP isoform and further comprises a carboxy-terminal di-lysine.

66. A method of claim 53, wherein the APP substrate comprises a human APP isoform and the determining step comprises measuring the production of amyloid alpha peptide (sAPPα).

68. A method of claim 66, wherein the method further comprises steps of:

(c) determining the level of hu-Asp1 α-secretase activity in the presence and absence of a modulator of hu-Asp1 α-secretase activity; and

(d) comparing the hu-Asp1 α-secretase activity in the presence and absence of the modulator, wherein modulators that increase hu-Asp1 α-secretase activity are identified as candidate Alzheimer's disease therapeutics.

69. A method of claim 67, wherein the method further comprises a step of treating Alzheimer's Disease with said candidate Alzheimer's disease therapeutic.

70. An Asp1 protease substrate peptide or fragment thereof, wherein said peptide comprises an amino acid sequence consisting of fifty or fewer amino acid, said amino acid sequence including the Asp1 cleavage site having the amino acid sequence GLALALEP.

71. The substrate of claim 68, wherein the substrate comprises a detectable label.

72. A method for assaying hu-Asp1 proteolytic activity comprising the steps:

(a) contacting hu-Asp1 enzyme with an Asp1 substrate according to claim 69 under acidic conditions, and

(b) determining the level of hu-Asp1 proteolytic activity.

73. A method according to claim 72, wherein the hu-Asp1 enzyme comprises a polypeptide produced in cell transformed or transfected with a polynucleotide comprising the nucleotide sequence that encodes hu-Asp1.

74. A method of claim 72, wherein the hu-Asp1 enzyme is purified and isolated from said cell.

72

~~75~~. A method according to claim ~~73~~, wherein the nucleotide sequence encodes a polypeptide that comprises the hu-Asp1 amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, wherein said fragment retains proteolytic activity.

~~76~~. A composition comprising an agent that modulates APP processing activity of hu-Asp1 according to the method of claim 26 in a pharmaceutically acceptable carrier.

~~77~~. A composition comprising an agent that modulates APP processing activity of hu-Asp1 according to the method of claim 37 in a pharmaceutically acceptable carrier.

~~78~~. A composition comprising an agent that modulates APP processing activity of hu-Asp1 according to the method of claim 48 in a pharmaceutically acceptable carrier.

add C37

Abstract of the Disclosure

The present invention provides the enzyme and enzymatic procedures for cleaving the β secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and cell isolates and assays. An enzyme that cleaves the α-secretase site of APP also is provided. The invention further provides a modified APP protein and associated nucleic acids, peptides, vectors, cells, and cell isolates, and assays that are particularly useful for identifying candidate therapeutics for treatment or prevention of Alzheimer's disease.